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Identification of soluble interleukin-4 receptor in rat glomerular epithelial cells[1]

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Abstract

Interleukin (IL)-4, a pleiotropic cytokine involved in many glomerular diseases, is regulated positively by membrane-bound IL-4R (mIL-4R) and negatively by soluble IL-4R (sIL-4R). Because natural sIL-4R has been documented only in mice, we undertook this study in rats to determine whether they, too, express sIL-4R, particularly in kidney cells. A pair of IL-4R primers was designed for this purpose and used in the polymerase chain reaction. As a result, sIL-4R was found not only in rats spleen cells but also in their glomerular epithelial cells (GEC). Sequence analysis revealed that the mRNA of rat sIL-4R has a 75-bp insert sequence. This insert generated a termination TGA codon upstream from the transmembrane region, resulting in formation of the sIL-4R. Subsequent screening of the kidney cDNA library enabled us to obtain the whole 3605-bp cDNA of sIL-4R; the full-length 3530-bp mIL-4R cDNA was also identified as a much longer sequence than previously published. Among the total 39 clones positive for IL-4R, two were confirmed as sIL-4R, and 37 clones were positive for mIL-4R. Next, the translated portion of sIL-4R cDNA was constructed into an expression vector, enabling us to obtain a recombinant sIL4R-myc fusion protein. By using this recombinant sIL-4R, we proved that sIL-4R can antagonize the IL-4-induced proliferation of spleen cells. Present study demonstrated that sIL-4R is expressed in kidney cells and antagonistically functional.

Keywords: Glomerular epithelial cell; Interleukin-4 receptor; Rat; Kidney

1. Introduction

Interleukin (IL)-4, produced mainly by activated T cells, has a broad spectrum of biological activities. This cytokine regulates such aspects of lymphocyte function as induction, activation and differentiation of B cells, suppression of B-cell apoptosis, promotion of precursor T helper cell differentiation into mature Th2 cells and inhibition of some proinflammatory cytokines production [1–4]. In addition to its immunomodulatory functions, IL-4 can also mediate murine pulmonary and human dermal fibroblast proliferation, cytokine synthesis and extracellular matrix production [5,6]. Moreover, recombinant rat IL-4 was found to enhance epithelial cell proliferation [7].

Up to now, evidence that IL-4 is involved in renal

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[1] The sequence data used in this paper have been submitted to the DDBJ/EMBL/GenBank Nucleotide Sequence Database under accession numbers AB015746 (sIL-4R) and AB015747 (mIL-4R).

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disease comes from several sources. In vivo, infiltration of IL-4-producing T cells is a common pathological feature in the renal diseases of humans and rats [8–19]. In vitro, IL-4 has stimulated synthesis of the extracellular matrix, caused the release of cytokines such as IL-6, IL-8 and MCP-1 by glomerular mesangial cells and glomerular epithelial cells (GEC) [20,21]. Moreover, IL-4 enhances the expression of vascular cell adhesion molecule-1 (VCAM-1) by GEC [22]. IL-4 also inhibits of proliferation of human mesangial cells [23].

IL-4 manifests its biological effects by binding to specific receptors on the cell surface. Mice have two kinds of IL-4 receptors: membrane bound IL-4R (mIL-4R) and soluble IL-4R (sIL-4R). Mature murine mIL-4R is a 140 kDa transmembrane glycoprotein containing extracellular, transmembrane and cytoplasmic domains. In contrast, sIL-4R has only the extracellular portion and completely lacks the transmembrane and cytoplasmic domains [1]. These two forms are encoded by distinct mRNAs, both derived from the same gene [1]. In sIL-4R, a 114-bp insert introduces a termination TGA codon that ends the open reading frame upstream of the predicted transmembrane region, resulting in a truncated form of the receptor [1]. The important similarity, though, is that mIL-4R and sIL-4R have the same affinity to IL-4; mIL-4R can combine with IL-4 and transmit signals across the plasma membrane, thus initiating the biological activities of IL-4 [24]. In contrast, sIL-4R retains high ligand-binding activity and acts as a competitive inhibitor of IL-4 binding to membrane receptors, thereby regulating the biological activity of IL-4 [3,25–29]. Until now, sIL-4R has been found only in mice, although mIL-4R cDNA has been documented in rats [30]. Even so, some researchers have concluded that the expression of sIL-4R is limited to murine species alone [31].

To resolve this issue, we designed a pair of IL-4R primers and conducted the polymerase chain reaction (PCR). Use of this technology identified sIL-4R in rats. We then screened the kidney cDNA library and obtained the entire cDNA of sIL-4R. This recombinant sIL-4R was then used to investigate the receptors effect on cell proliferation induced by IL-4 in spleen cells. The results confirmed that sIL-4R is capable of specifically neutralizing the biological effects of IL-4. This is the first report of sIL-4R in the rat and of sIL-4R expression in renal cells.

2. Materials and methods

2.1. Cells

An established cell line of rat GEC was used and cultured in a 1:1 fresh mixture of K1 medium and 3T3 fibroblast-conditioned medium. K1 medium contains Ham F-12 (Life Technologies, Grand Island, NY), Dulbecco's modified Eagle's medium (DMEM), Nu Serum (Collaborative Research, Bedfold, MA), L-glutamine, ITS (insulin, transferrin, selenium), Hepes and antibiotics.

Wistar rat MC, NRK (normal rat kidney fibroblast), Swiss mouse 3T3 fibroblast and COS-7 monkey kidney cells were obtained from American Type Culture Collection (ATCC) and maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine, L-arginine, L-asparagine, sodium pyruvate and Hepes.

2.2. Animals and tissue preparation

Male Wistar rats, 5 weeks of age, were purchased from SLC Co., Japan and maintained in our animal facility. Specimens of various organs were obtained and snap-frozen in liquid nitrogen, then stored at −70°C for further use.

2.3. Isolation of total RNA

Total RNA was isolated from cultured cells and tissues using ISOGEN (Nippon Gene, Japan) as recommended by the manufacturer. The precipitated RNAs were dissolved in diethyl pyrocarbonate (DEPC)-treated water containing 0.5% sodium dodecyl sulfate (SDS). Quantification was done by spectrophotometer.

2.4. Reverse transcription of RNA

cDNA synthesis was carried out in 100 μl of a reaction mixture containing 2 μg of total RNA, 20 μl 5×RT buffer, 10 μl 2.5 mM dNTPs, 1 μl 20 μM

Oligo dT15 Primer, 1 μl ribonuclease inhibitor. After heating at 68°C for 5 min then icing, 1 μl of Moloney murine leukemia virus reverse transcriptase (RT) was added and incubated at 42°C for 45 min. This was followed by heat-inactivation of the RT at 68°C for 5 min.

2.5. PCR amplification of IL-4R mRNA

PCR amplification was performed in a 25 μl reaction containing 1 μl of reverse-transcribed RNA, 2.5 μl 10×PCR buffer (Toyobo), 1.5 μl 25 mM $MgCl_2$, 1 μl 2.5 mM dNTP, 1 μl 25 μM primer and 1 unit rTaq DNA polymerase (Boehringer Mannheim, Germany). Samples were overlaid with mineral oil and heated at 94°C for 3 min, followed by 30 cycles (denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min) and at 72°C for 5 min. The PCR products were visualized on a 2% agarose gel by ethidium bromide staining. The primers used in this study were as follows. (1) for IL-4R: 5′-AGT CAA CAC CCT AAC ATC AGG G-3′ (sense) and 5′-GGC CTC AAA CAG CTC CAT ACA G-5′ (antisense). Two bands were obtained, the 547-bp band representing a mIL-4R message, and the 622-bp band indicating a sIL-4R message. (2) For the rat insert sequence (RIS) of soluble IL-4 receptor: 5′-CAA ATA ATG TCA ATC TG-3′ (sense) and 5′-CAG CTC CAC TGC TCT GT-3′ spanning a 75-bp fragment (antisense). (3) For actin, used as control: 5′-ATG TAC GTA GCC ATC CAG GCT-3′ (sense) and 5′-ATT GCC GAT AGT GAT GAC-3′ spanning a 363-bp fragment (antisense).

2.6. Sequencing of the PCR products

The PCR products were electrophoresed and the two bands were purified with a DNA Purification kit (Bio-Rad Laboratories, USA). The purified fragments were cloned into a TA vector according to the standard protocols using a TA Cloning kit (Invitrogen, USA) In brief, 2 μl purified PCR products, 1 μl 10-fold ligase buffer, 2 μl TA Vector, 1 μl T4 ligase and 4 μl sterile water were allowed to incubate at 14°C overnight.

Recombinant plasmids were transfected to competent cells, then amplified and identified by *Eco*RI restriction digestion. Nucleotide sequence analysis was conducted by fluorescent dye primers (T7 and reverse primers) using Thermo Sequenase fluorescent-labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Life Science, UK) according to the manufacturer's instructions. Automatic DNA sequencing was performed with DNA Sequencer. To exclude PCR artifacts, all IL-4R sequences were determined in three separate clones.

2.7. Isolation of cDNA and determination of the cDNA sequence

The cDNA library was constructed in the λgt10 vector with mRNA from Sprague–Dawley rat kidney. The library was screened first with the PCR product of IL-4R (622 bp) and then with RIS (75 bp) as mentioned above. The probes were labeled by a random primer method using [α-^{32}P]dCTP and the Klenow enzyme (Boehringer Mannheim). The hybridization solution was 50% formamide, 5×SSC (1×SSC: 0.15 M sodium chloride, 0.015 M sodium citrate), 8×Denhardt's solution (1×Denhardt's solution: 0.02% Ficoll, 0.02% polyvinyl pyrolidone, 0.02% bovine serum albumin), 50 mM phosphate buffer (pH 6.5), and 250 μg/ml heat-denatured herring sperm DNA. The filters were hybridized with labeled probes overnight at 42°C and washed twice in 2×SSC/0.1% SDS at room temperature for 5 min, and subsequently washed twice in 0.1×SSC/0.1% SDS at 65°C for 20 min, then exposed to Kodak XAR film with intensifying screen at −70°C. After the third screening, single positive clones were selected and *Not*I/*Sal*I inserts were cloned into pBluescriptSK+ vector (Stratagene, USA). Since the insert cDNA is about 4 kbp, small fragments were obtained by digestion with several special restriction enzymes, then subcloned into pBluescriptSK$^+$ again. The DNA sequence was determined in both directions by the dideoxy chain termination procedure using fluorescence-labeled T7 and reverse primers as cited above. DNA sequence assembly and comparison were carried out by use of the GENEYTX-MAC program.

2.8. Construction and expression of sIL-4R fusion protein

The translation part of sIL4R cDNA was trimmed between nt 158 and nt 865 with synthetic *Bam*HI and *Eco*RV adapter by PCR from the full-length sIL-4R cDNA (pBSSK/RS36). The upstream sense primer containing the *Bam*HI restriction endonuclease site is 5′-GGG GAT CCA GGC ACC TTT GTG T-3′ and the downstream antisense primer containing the *Eco*RV site is 5′-GGA TAT CAG ATT GAC ATT ATT TGG-3′. The amplified product was digested with *Bam*HI/*Eco*RV and subcloned into the mammalian expression vector pcDNA3.1C (Invitrogen, The Netherlands) to generate pcDNA3.1C/sIL4R and subsequently confirmed by sequence analysis.

The constructed pcDNA3.1C/sIL4R was used to transfect a subconfluent layer of COS-7 cells by TfxTM-20 Reagent for the Transfection of Eukaryotic Cells kit (Promega, USA) according to the recommended instructions. The cells were then grown in culture to permit transient expression of the inserted sequence. pcDNA3.1C vector alone and pcDNA3.1-LacZ (Invitrogen) were also used as controls. After 3 days, supernatants were harvested and the recombinant fusion protein were purified by Xpress System ProBond Resin (Invitrogen) according to the recommended instructions. The recombinant fusion proteins were dialyzed with PBS and concentrated by molecular cut filter, then quantitated.

2.9. Cell proliferation assays

A splenocyte proliferation assay was used to determine IL-4 bioactivity [32]. Spleens were aseptically removed from Wistar rats, and the cells were isolated and filtered through Sephadex G-10 (Pharmacia, Sweden) to remove adherent cells as previously described [33]. Splenocytes (5×10^4 cells/well) were cultured in triplicate in 96-well microtiter plates in 10% FCS/DMEM with 10 nM PMA and 50 μM 2-ME. These cells were treated with 10 ng/ml recombinant rat IL-4 (R & D Systems, USA) in the presence of various concentrations of recombinant rat sIL-4R-myc or recombinant LacZ-myc and cultured at 37°C. [^{3}H]Thymidine (1 μCi/well) was added during the last 6 h of a 24-h incubation period, after which the cells were harvested onto glass-fiber filters and examined for thymidine uptake by liquid scintillation counter.

3. Results

3.1. Expression of sIL-4 receptors in GEC

To explore whether natural sIL-4R is expressed in rats, we devised a pair of primers spanning the receptor of extracellular and intracellular domains containing the transmembrane region. The primer sequences were deduced from mouse IL-4R primers [5] and the rat IL-4R cDNA sequence (EMBL accession number X69903). We assumed that if the rat system includes sIL-4R with an inserted sequence like that in the mouse, PCR would amplify bands of sIL-4R and also mIL-4R. On that premise, the RT–PCR was conducted on cultured rat MC, GEC, NRK and normal kidney tissue. Spleen cells and COS-7 cells were also used as positive and negative controls respectively. According to the results of PCR, GEC and normal kidney tissues had two bands (Fig. 1). However, the large band was very faint in were also present in the control spleen cells, but none was apparent in COS-7 cells. Negative controls without template cDNA or reverse transcriptase were also included but contained no bands (not shown). Amplification of the housekeeping gene, actin, was used as a control for RNA integrity and PCR. The expression of actin was equally expressed

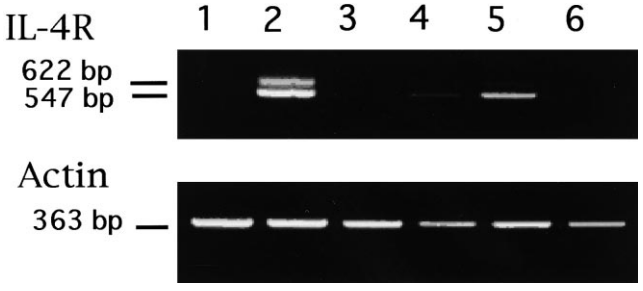

Fig. 1. RT–PCR analysis of rat IL-4R mRNA expression. Samples were mesangial cells (lane 1), GEC (lane 2), NRK (lane 3), kidney tissue (lane 4), spleen cells (lane 5), and COS-7 cells (lane 6). The band at 622 bp corresponds to the sIL-4R message and the band at 547 bp stands for mIL-4R. The housekeeping gene, actin (363 bp), was also amplified as a positive control for intact RNA and efficiency of RT–PCR.

AGTCAACACCCTAAACATCAGGGGTGCGCTATAGGGCACGTGTGAGGGTC

TTGTCCCAGAGCTTCCCTGGCATCTGGAGTGAGTGGAGTCCCAGCATCAC

GTGGTACAACCCAAATAATGTCAATCTGTGACTGAATGACCTTGGGGGCT

ProAsnAsnValAsnLeu***

CCAGTGGTGAGGAGAGCACACAGAGCAGTGGAGCTGACTTCCAGCTGCCC

CTGCTGCAGCGCCTCCCGCTGGGTGTCAGCATCTCCTGCATCTGCATCCT

ATTGTTTTGCCTGACCTGTTACTTCAGCATTATCAAGATTAAGAAGATAT

GGTGGGACCAGATTCCCACTCCAGCACGCAGTCCCTTAGCAGCCATCATC

ATTCAGGACACGAAGGTGTCCCTCTGGGAGAAGCAGACCCGAAGCCAGGA

GTCAACCAAGAGCCGACACTGGAAGACTTGTCTAACCAAGCTGCTGCCCT

GCTTGCTGGAGCATAGAGTGAGGAAAGAGAGAGAATCCCCGAAGGCTGCC

AAAACCAAGCCTCTCCAGAGTCCTGAAAAAGCAGGCTGGTATCCTGCGGA

GGTCAGCAGGACCGTCCTCTGGCCAGAGAACGTTCATGTCAGTGTGGTGC

GCTGTATGGAGCTGTTTGAGGCC

Fig. 2. Nucleotide sequences of the amplified RT–PCR products. The whole nucleotide sequence represents a 622-bp band corresponding to sIL-4R message. A 75-bp message in black box is inserted into mIL-4R with 547 bp. Six amino acids are newly generated in this insertion. The PCR primer sites are marked by dotted underline and the transmembrane region is underlined.

in each sample (Fig. 1). These results denoted the existence of sIL-4R in the rat.

3.2. Sequence analysis of rat PCR products

To confirm that the PCR products were rat IL-4R, the two bands amplified in glomerular epithelial cells were isolated and purified separately, then subcloned into TA cloning vectors. When the sequences were determined and compared with the published rat IL-4 receptor cDNA sequence, one band that had been considered as a membrane-bound receptor was completely identical to the predicted, published sequence, showing that the primer-amplified fragment was rat IL-4R. Meanwhile, another band thought to represent the soluble receptor had an exceptionally short sequence that differed from the long one only by a 75-bp insert (black boxed in Fig. 2). The nucleic acid sequence of inserted fragment varied from that of mouse [1] by 6 amino acids and the termination site. This termination site induced formation of sIL-4R.

3.3. Isolation of full-length rat IL-4R cDNA

Since RT–PCR showed, and sequencing confirmed, that rats express sIL-4R, a rat kidney cDNA library was screened. For the first screening, we used the 622-bp IL-4R PCR product as a probe that recognized both sIL-4R and mIL-4R. Approximately 150 000 plaques were screened, from which 39 independent clones were identified as positive. After a second screening using the 75-bp insert fragment as a probe that hybridized only to sIL-4R, two of 39 clones were positive and of the same size. Finally, two clones were selected as representative of the soluble and membrane-bound forms. The fragments were digested, subconstructed into the *Not*I/*Sal*I site of pBluescriptSK+ vector and designated as pBSSK/RS36 and pBSSK/RM4, respectively. Because the cDNAs extended about 4 kbp in length, several restriction enzyme sites were used (Fig. 3), and the resulting small fragments were again subcloned and sequenced. The full-length mIL-4R cDNA (pBSSK/RM4) was 3530 bp containing the ATG codon and a poly(A) stretch much longer than that reported for this sequence in the rat. This cDNA has an open reading frame encoding 801 amino acids flanked by 178 bp of 5′ and 943 bp of 3′ noncoding sequence. Clone pBSSK/RS36 was distinguished from pBSSK/RM4 by having a 75-bp insertion at nucleotide number 849 containing a termination codon TGA (Figs. 2 and 3). This insertion

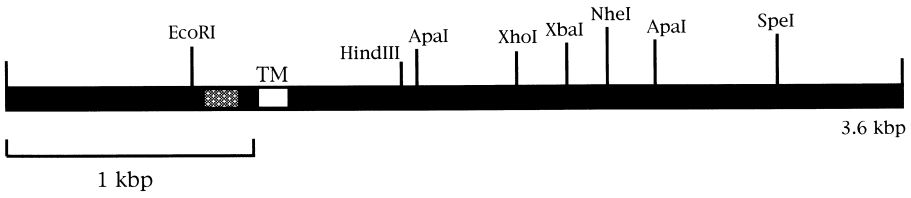

Fig. 3. Restriction maps and schematic representation of sIL-4R. The open box represents the transmembrane region; the shaded box stands for insert fragments. The boxed nucleotide acid sequence shows the insertion with a termination TGA codon.

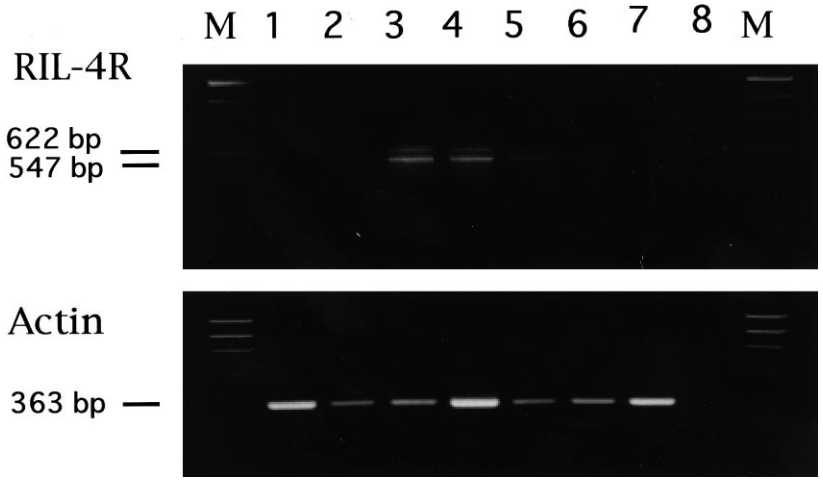

Fig. 4. Distribution of rat sIL-4R by RT–PCR analysis. Samples were from brain (lane 1), heart (lane 2), lung (lane 3), liver (lane 4), kidney (lane 5), spleen (lane 6), muscle (lane 7), and reagent control without a template (lane 8). The band at 622 bp corresponds to the sIL-4R message, and the band at 547 bp stands for mIL-4R. The housekeeping gene, actin (363 bp), was also amplified as a positive control for intact RNA and efficiency of RT–PCR.

generated additional six amino acids. The resulting protein was 229 amino acids in length and represented a potential soluble form of the IL-4 receptor in rats.

3.4. Tissue distribution of sIL-4R

To define the distribution of sIL-4R in vivo, we again conducted the RT–PCR, this time with tissues from brain, heart, lung, liver, kidney, spleen, and muscle by using the relevant mRNA. Recapitulating its appearance in the kidney and spleen, sIL-4R was also expressed in the lung and liver but not in the brain, heart or muscle (Fig. 4).

3.5. Expression and purification of sIL-4R fusion protein

To investigate the possible function of sIL-4R, sIL4R-myc fusion proteins were generated. The translation portion of sIL-4R cDNA was inserted into a pcDNA3.1C mammalian expression vector with the myc epitope and polyhistidine tag sequence (Fig. 5A). Plasmids were constructed then transfected into COS-7 cells. The resulting recombinant proteins were purified with resin by polyhistidine tag. Subsequent Western blot analysis (Fig. 5B) showed that COS-7 cells transfected with pcDNA3.1C/sIL4R secreted sIL-4R-myc fusion proteins with a molecular size of about 45 kDa. Cells transfected with pcDNA 3.1LacZ also secreted LacZ-myc fusion proteins; however, no myc fusion proteins were detectable in cells transfected with pcDNA 3.1C vector alone.

3.6. Inhibition by sIL-4R on splenocyte proliferation induced by IL-4

To examine the effect of sIL-4R on the biological activity of IL-4, splenocyte proliferation in response to IL-4 stimulation was used as originally described by Noble and Kemeny [32]. When combined with PMA, IL-4 induced the proliferation of spleen cells in a dose-dependent manner (Fig. 6A). When the cells were cultured in 10 ng/ml IL-4 with incremental increases of recombinant sIL-4R, thymidine incorporation decreased dose-dependently (Fig. 6B). In contrast, recombinant LacZ had no inhibitory effect on the cell proliferation induced by IL-4. In each group, the difference between outcomes with sIL-4R and LacZ was statistically significant. Clearly, sIL-4R neutralized the biological effects of IL-4 in vitro.

4. Discussion

As documented here, we have cloned and determined the entire sequence of rat IL-4R cDNA including sIL-4R and mIL-4R. To our knowledge,

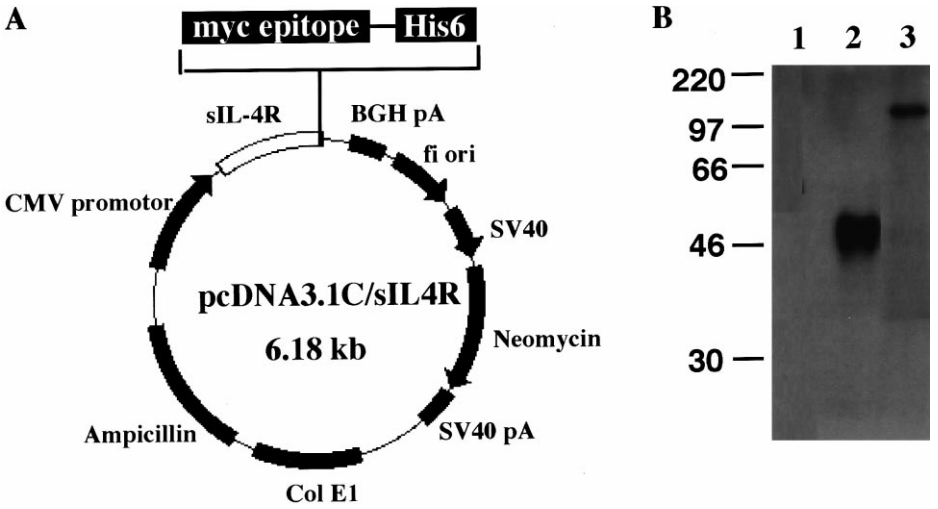

Fig. 5. (A) Map of the constructed rat sIL-4R expression vector (pcDNA3.1C/sIL-4R). (B) Western blot analysis of recombinant protein. Culture media from COS-7 cells transfected with pcDNA3.1C vector alone (lane 1), pcDNA3.1C/sIL-4R (lane 2) and pcDNA3.1 LacZ (lane 3) were purified by histidine-affinity resin; eluted samples were subjected to SDS–PAGE followed by immunoblotting with anti-myc monoclonal antibody and detected by ECL. Molecular mass is indicated in kDa.

this is the first report of sIL-4R in the rat. When we then prepared recombinant sIL-4R and studied its effect on biological activity, our results confirmed that sIL-4R can neutralize IL-4-stimulated proliferation of spleen cells. After a first screening of the RT–PCR product from rat kidney cells, 39 clones of IL-4R cDNA were obtained. A second screening with an RIS probe, which hybridized only to sIL-4R, yielded two clones. Therefore sIL-4R accounts for about 5% of IL-4R mRNA, consistent with Mosley's observation of 5–10% in mice [1]. At least in normal kidneys, the expression of mIL-4R is thus preferential to that of sIL-4R, accounting for the very weak band of sIL-4R we found after performing RT–PCR.

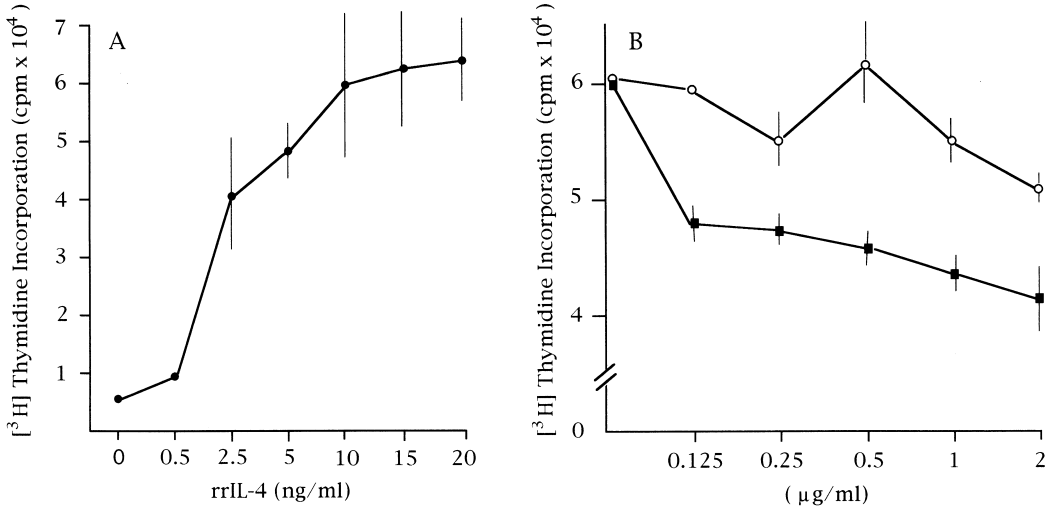

Fig. 6. [^{3}H]Thymidine-incorporation assay of splenocyte proliferation. (A) Splenocytes in combination with 10 nM PMA were cultured with increasing concentrations of rat recombinant IL-4. (B) PMA-activated splenocyte were cultured with 10 ng/ml IL-4 in the presence of various recombinant sIL-4R-myc (●) and LacZ-myc (○). Cells were cultured in triplicate for 24 h and pulsed with [^{3}H]thymidine. All data are expressed as means ± S.E. Statistical differences were assessed by Scheffe test in each group. Each group showed a significant difference ($P < 0.001$).

On that basis, we speculated that, under some pathological conditions, the normally limited expression of sIL-4R may be upregulated and act to restrain the immunological injury that IL-4 can cause. IL-4 is not the only cytokine with two forms of receptors, membrane-bound and soluble. However, unlike other soluble cytokine receptors that are formed by shedding of membrane receptors, such as sIL-2R and sTNF-R [34,35], the murine sIL-4R is transcribed from an alternative message, quite separate from that encoding the membrane form [1]. Murine sIL-4R has a short insert that introduces a termination codon upstream from the transmembrane region. This termination site results in secretion of the receptors truncated form [1]. Since the sIL-4R binds IL-4 with high affinity, it can act as a neutral antagonist of IL-4 activity, competing with the mIL-4R on target cells for the binding of IL-4. Although Richter et al. cloned partial rat IL-4R, until now this form of the cytokine receptor had not been found as a natural constituent of rats [30]. Furthermore, Idzerda, who tested humans for IL-4R, concluded that only murine species secreted IL-4R [31].

In this study, to seek sIL-4R in rats, we designed a pair of primers spanning the extracellular and intracellular domains of the IL-4R. The results of PCR testing clearly demonstrated that rat GEC and splenic cells express both sIL-4R and mIL-4R. Successful cloning and subsequent sequencing of the full-length IL-4R cDNA showed 3605 bp in sIL-4R and 3530 bp in mIL-4R. The latter fragment, mIL-4R, was longer than the 2450 bp reported by Richter et al. [2], perhaps because of an underestimation.

The unique difference between the two forms of rat IL-4R is the 75-bp insertion sequence in sIL-4R at nucleotide number 849 upstream of the transmembrane region. In comparison to rats, mice have an sIL-4R insertion of 114 bp [1]. However, both these sequences contain a termination TGA codon that introduces six novel amino acids at the C-terminus. This finding suggests that formation of the insert was evolutionary event with biological significance.

Although sIL-4R lacks a transmembrane region and cytoplasmic domain, it still has high affinity to IL-4. Studies done in vivo and in vitro have demonstrated that sIL-4R can act as competitive inhibitor of IL-4 binding to membrane receptors, thereby antagonizing the cytokines biological activity. For example, Mosley et al. found that sIL-4R specifically blocked IL-4-induced CTLL cell proliferation [1]. Similarly, by generating and using a sIL4R-myc fusion protein, we found that recombinant sIL-4R inhibited IL-4-induced splenocyte proliferation. In both situations, sIL-4R neutralized the biological effects of IL-4.

In the present study, cultured rat GEC expressed large amounts of sIL-4R, and whole sIL-4R was retrieved after screening cDNA from a rat kidney library. Our experiments showed that the expression of sIL-4R is much lower in whole kidney tissue than in cultured GEC (Figs. 1 and 4), which may account for our finding only two sIL-4R clones from 39 IL-4R clones in the present study. Furthermore, the IL-4R in kidney tissue is mostly in the membrane form (94.9%). Although the precise function of sIL-4R is still conjectural, much evidence has implicated IL-4 in the pathogenesis of both human and experimental glomerulonephritis [8–19]. We found that Jak3, which is activated only by IL-4 in GEC, is upregulated in IgA nephropathy and accompanies deteriorating glomerular function [10]. In addition, by using in situ hybridization, Furusu and Miyazaki demonstrated that the expression of IL-4 correlated positively with the degree of mesangial hypercellularity and expansion of the extracellular matrix [19]. These reports suggested that the expression and function of IL-4R are critical in glomerulonephropathy. Quite possibly, sIL4-R is expressed in the renal tissue other than GEC; for example, the latter authors reported that the IL-4R was expressed constantly in mesangial cells. However, we did not detect either type of IL-4R in cultured mesangial cells (Fig. 1), perhaps because that trait was lost during culture. Technical improvement of in situ hybridization or antibody reactive with sIL-4R should resolve this issue.

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